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Site-Specific Recognition of Dipeptides Through Non-Covalent Inter-Ligand Interactions for the Hydrolysis of Dipeptide to Amino Acid Ligands Mediated by Ternary Cobalt(III) Complexes

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Molecular recognition of dipeptide (DP) compounds is performed with a ternary cobalt(III) complex containing the tripodal tetradentate ligand bis-*N*,*N*-carboxymethyl-L-phenylalanine (H_3bcmpa). The coordination structure of the complex [Co(bcmpa)(dp)]$^-$ (**1**–**14**) was spectroscopically assigned by UV/Vis, CD, and ^{1}H NMR spectroscopic methods. Site-specific recognition of the dipeptide on the ternary complex is achieved by various weak, non-covalent, inter-ligand interactions, such as hydrogen bonding, steric repulsion, and electrostatic interaction, etc. Some dipeptides having C-terminal aromatic side-chains also demonstrate inter-ligand CH–π interactions between the aromatic ring and the α-hydrogen of bcmpa. Coordination of the N-terminal nitrogen atom of the dipeptide to cobalt at the *trans*(N)-position to the tertiary nitrogen of bcmpa in the octahedral geometry provides an aminopeptidase model. Under slightly alkaline conditions, the dipeptide ligand of some of these complexes is cleaved to give the ternary complex, [Co(bcmpa)(aa)]$^-$ (aa = amino acidato). The rate for the hydrolysis of dipeptide decreases upon increasing the steric bulkiness of the C-terminal side-chain. In the cases of the ternary complexes [Co(bcmpa)(gly-phe)]$^-$ (**6** and **11**), which have C-terminal L- or D-phenylalanine, the hydrolysis of gly-phe to gly was completely prevented. On the other hand, the ternary cobalt(III) complexes [Co(bcmga)(gly-phe)]$^-$ (**18** and **19**), where H_3bcmga (bis-*N*,*N*-carboxymethyl-L-glutamic acid) is the more hydrophilic tripodal ligand, give a small amount of the corresponding cleavage product [Co(bcmga)(gly)]$^-$. The hydrophobic sphere generated around the metal complex in **6** and **11** regulates approach of OH^- ion to the amide carbonyl group. Multi-site interactions mediated on the ternary complexes are available for the design of an artificial hydrolysis enzyme. This is the first report describing the substrate-specific cleavage of peptides using a simple enzyme model complex.

Introduction

Various models for the enzyme reaction have been investigated in the course of studying biomimetic catalysis.[1,2] Hydrolysis of the peptide bond is usually carried out in strongly acidic or alkaline conditions as the reaction in neutral aqueous media is very slow. Although hydrolysis of a gly-val compound under non-catalytic conditions requires more than 200 years,[3] the presence of an enzyme accelerates the process remarkably.[4] Kimura has reported the sequential hydrolysis of peptides in the presence of a [Co(trine)(OH)(H_2O)]$^{2+}$ complex, in which various oligopeptides were cleaved to give amino acids.[5] At around the same time, Bentley et al. also reported cleavage of peptide ligands mediated by the [Co(trine)(OH)(H_2O)]$^{2+}$ complex.[6] Using this cobalt(III) complex, moreover, Boreham, et al. have reported a mechanistic investigation of the intermediate of the amide cleavage reaction.[7] In these previous studies using cobalt(III) complexes, the selectivity of the cleavage reaction was not discussed. Using cobalt(III) complexes combined with peptide-hapten, Iverson and Lerner reported the sequence-specific hydrolysis of the gly-phe bond.[8] Unlike these previous reports, exopeptidase activity was demonstrated by the regioselective cleavage of lysozyme using [Co(NH_3)$_4$X(H_2O)] (X = OH or H_2O) complexes as artificial metallopeptidases.[9] Recently, Chae et al. have reported that a cobalt(III) cyclen complex containing a diamide moiety shows an *endo*-selective cleavage activity for peptides.[10] However, there have been few reports demonstrating selectivity for the cleavage of peptides mediated by cobalt(III) complexes. Since Kostic et al. first reported that platinum(II) or palladium(II) ions show catalytic activity for the hydrolysis of peptides,[11] there have been some examples reported of the selective cleavage of peptide compounds. These systems are considered to be an artificial enzyme because the peptide itself becomes a ligand due to coordination of the sulfur or nitrogen atom, for example with peptides including cysteine (cys), methionine (met), or histidine (his).[12] Although a detailed mechanism for the

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hydrolysis reaction is not yet clear, the carbonyl group of the amide bond to a neighboring cys, met, or his residue was found to be selectively hydrolyzed under mild conditions when platinum(II) complexes containing two substitutionally labile sites were used.[13]

With a view to investigating metalloenzymes, transition metal complexes provide an attractive tool for constructing an artificial enzyme model of peptidase through various weak, non-covalent interactions.[14–18] A combination of non-covalent interactions observed at or near the active site of the enzyme causes high efficiency and specificity for an enzymatic reaction, which is essential for biological reactions involving substrate recognition.[19] For example, various non-covalent interactions, such as hydrogen bonding, hydrophobic interactions, steric repulsions, and electrostatic interactions, have been clearly demonstrated at the active site of the enzyme–substrate complex formed from aminopeptidase and bestatin.[20] Moreover, an interesting approach to delineate the non-covalent bonds between chromium(III) complexes containing a nitrilotriacetate framework and a protein surface has been described that uses NMR and CD spectroscopy.[21] We have hitherto been interested in the construction of molecular recognition models for enzyme–substrate complexes,[22–25] in which the cobalt(III) complex $[Co(bcmpa)(aa)]^-$, which contains the tripodal tetradentate ligand bis-*N*,*N*-carboxymethyl-L-phenylalanine (H_3bcmpa), is considered as an enzyme model, with a didentate amino acid (AA) as substrate. Various amino acids were found to coordinate to Co^{III}-bcmpa in a *trans*(N) configuration rather than a *cis*(N) one.[22,24] It is very interesting that such a simple metal complex demonstrates the coordination-selective recognition of amino acids through various non-covalent interactions between bcmpa and the amino acid moiety.[17] In addition, this Co^{III}-bcmpa complex self-assembled on a Au electrode also becomes a new promoter electrode that recognizes the electron-transfer site in enzymes.[26]

In this study, we report the synthesis of ternary cobalt(III) complexes $[Co(bcmpa)(dp)]^-$ with a dipeptide (DP) ligand and characterize the inter-ligand interactions for the site-selective recognition of dipeptides on the complexes spectroscopically in solution. We also investigate the cleavage reaction of the dipeptide ligand in slightly alkaline solution. Selective hydrolysis of the dipeptide ligand mediated by the ternary cobalt(III) complexes is caused by the various non-covalent inter-ligand interactions, which allow this complex to be considered as an aminopeptidase model.

Abbreviations

gly-gly: glycylglycine; gly-ala: glycylalanine; ala-gly: alanylglycine; gly-leu: glycylleucine; leu-gly: leucylglycine; gly-phe: glycylphenylalanine; phe-gly: phenylalanylglycine; gly-val: glycylvaline; gly-phg: glycylphenylglycine; gly-β-ala: glycyl-β-alanine; gly-Mala: glycyldimethylglycine.

Results and Discussion

Coordination Structure of the Ternary Cobalt(III) Complexes with Dipeptide Ligands

The complexation of $K_2[Co(bcmpa)(CO_3)]$ with various dipeptide compounds in neutral aqueous solution in the presence of active charcoal gave two negatively charged complexes after QAE Sephadex exchange column separation with aqueous KCl solution. One of the separated bands was found to contain the orange-colored complex K[Co(bcmpa)(dp)] (**1**–**14**), whereas the other contained the pink-colored complex K[Co(bcmpa)(aa)]; the ratio of the former to the latter was about 10:1. The UV/Vis spectra of the main band containing cobalt(III) complexes with a dipeptide ligand are very similar to those of the previously reported complexes *trans*(N)-$[Co(bcmpa)(gly)]^-$ and *trans*(N)-$[Co(bcmpa)(leu)]^-$.[22] The first absorption band at $19.6–19.8 \times 10^3$ cm^{-1} ($\log\varepsilon$ = 2.15–2.18) with a shoulder at about 16×10^3 cm^{-1} ($\log\varepsilon$ = about 1.3) of complexes **1**–**14** indicates that two nitrogen atoms occupy the *trans* position in an octahedral geometry.[24,27,28] Their second absorption bands are found at about $26.2–26.4 \times 10^3$ cm^{-1} ($\log\varepsilon$ = 2.17–2.19). On the basis of the spectral analogy to *trans*(N)-$[Co(N)_2(O)_4]^-$, which is supported by the X-ray single crystal analysis of *trans*(N)-$[Co(bcmpa)(gly)]^-$, *trans*(N)-$[Co(bcmpa)(leu)]^-$, and *trans*(N)-$[Co(bcmle)(phe)]^-$,[22,23] we consider that the *trans* position to the bcmpa nitrogen in the $[Co(bcmpa)(dp)]^-$ complex is occupied by the N-terminal amine nitrogen and the *cis* position by the amide carbonyl oxygen atom.[17,22] The compounds included in the pink-colored minor band were identified as *trans*(N)-$[Co(bcmpa)(aa)]^-$ complexes on the basis of the spectral analogy of their previously reported analogs.[22,24] When the complexation with $K_2[Co(bcmpa)(CO_3)]$ and dipeptide was carried out at higher pH, the yield of the pink-colored band increased and that of the orange-colored one decreased. These findings indicate that complex formation between the dipeptide and $[Co(bcmpa)(CO_3)]^{2-}$ is accompanied by hydrolysis of the amide bond. Moreover, the –1 charge of the reaction product, as determined by the column separation behavior, reveals that the amide carbonyl group of the dipeptide coordinates to the metal center without deprotonation. The hydrogen atom attached to the amide nitrogen of *trans*(N)-$[Co(bcmpa)(dp)]^-$ is not removed in neutral or weakly alkaline media under complex formation conditions.

The character of the ternary cobalt(III) complexes consisting of the optically active bcmpa and dipeptide was also investigated by recording their CD spectra.[27,29] The three positive CD peaks of the *trans*(N)-$[Co(bcmpa)(gly\text{-}gly)]^-$ complex **1** observed at 16.2, 19.6, and 26.0×10^3 cm^{-1} are very similar to those of *trans*(N)-$[Co(bcmpa)(gly)]^-$.[24] Two dipeptide complexes, namely $[Co(bcmpa)(leu\text{-}gly)]^-$ (**5**) and $[Co(bcmpa)(phe\text{-}gly)]^-$ (**7**), show positive CD peaks at 16.7, 18.7, and 26.4×10^3 cm^{-1} and a negative one at 20.9×10^3 cm^{-1}, respectively, very similar to complexes of $[Co(bcmpa)(leu)]^-$ with coordinated didentate optically active amino acids.[22,24] Moreover, the intensity of the posi-

tive CD peaks of **5** and **7** is relatively strong in comparison with those of [Co(bcmpa)(gly-ala)]$^-$ (**2**), [Co(bcmpa)(gly-leu)]$^-$ (**4**), and [Co(bcmpa)(gly-phe)]$^-$ (**6**). Enhancement of the CD intensity indicates the coordination of the asymmetric amino acid moiety of the dipeptide to the cobalt(III) ion. As shown in Figure 1, the differential CD spectrum between [Co(bcmpa)(gly-D-ala)]$^-$ (**10**) and [Co(bcmpa)(gly-gly)]$^-$ (**1**) has a perfectly antipodal form, as does that between **2** and **1**.[29] Similar differential CD spectra were observed in the complexes coordinated by gly-L-phe (**6**) and gly-D-phe (**11**). The characteristic CD spectra of complexes **1**–**14** reveal that the N-terminal nitrogen atom of the dipeptide coordinates to the cobalt ion in a *trans*(N) manner.

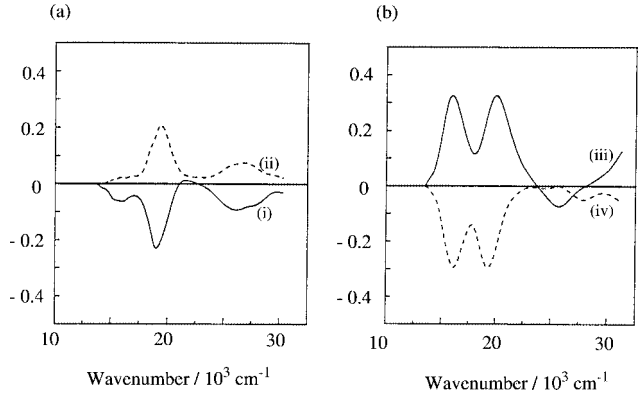

Figure 1. Differential CD spectra of the cobalt(III) complexes with dipeptide ligands. (a) Effect of C-terminal alanine: (i) [Co(bcmpa)(gly-ala)]$^-$ (**2**) – [Co(bcmpa)(gly-gly)]$^-$ (**1**); (ii) [Co(bcmpa)(gly-D-ala)]$^-$ (**10**) – [Co(bcmpa)(gly-gly)]$^-$ (**1**). (b) Effect of C-terminal phenylalanine: (iii) [Co(bcmpa)(gly-phe)]$^-$ (**6**) – [Co(bcmpa)(gly-gly)]$^-$ (**1**); (iv) [Co(bcmpa)(gly-D-phe)]$^-$ (**11**) – [Co(bcmpa)(gly-gly)]$^-$ (**1**).

The coordination structure of the diamagnetic cobalt(III) complex in solution was investigated by ^{1}H NMR spectroscopy. The characteristic ^{1}H NMR spectra of dipeptide complexes **1**–**14** are summarized in Table 1, where they are compared with those of [Co(bcmpa)(aa)]$^-$ previously identified.[24] In [Co(bcmpa)(gly-gly)]$^-$ (**1**), three pairs of AB patterns are observed in the methylene region. One of these (δ = 3.98 and 4.63 ppm, J = 18.0 Hz) was assigned to the R-ring hydrogens of bcmpa.[30] The G-ring hydrogens of bcmpa at δ = 3.20 and 4.13 ppm show a slightly smaller coupling constant of 16.8 Hz.[31] The others at δ = 4.24 and 4.25 ppm (J = 17.3 Hz) were identified as the N-terminal methylene hydrogens of the coordinating glycine moiety. The ABX pattern demonstrated by two benzyl hydrogens at δ = 3.51 (J = 15.1 and 10.6 Hz) and 3.64 ppm (J = 15.1 and 4.8 Hz) and the adjacent α-hydrogen at δ = 4.90 ppm (J = 10.6 and 4.8 Hz) is derived from the Gs-ring of bcmpa.[30] The singlet observed at δ = 3.82 ppm was identified as the C-terminal methylene hydrogens of the glycine moiety. The aromatic hydrogens of the bcmpa side-chain appear at δ = 7.4–7.6 ppm. As shown in Table 1, the chemical-shift values of the α-hydrogens of the N-terminal amino acid residue appear slightly downfield in comparison with those of the C-terminal one except for complexes **9** and **12**, which contain a C-terminal phenylglycine moiety. The above complex formation shifts of the N-terminal amino acid moiety indicate N-terminal coordination of the dipeptide to cobalt with formation of an N–O chelate. Taking account of the UV/Vis, CD, and ^{1}H NMR spectral investigations, the coordination structure of the dipeptide complex was determined to be that shown in Figure 2 (a), although other structures (b–f) are also possible.

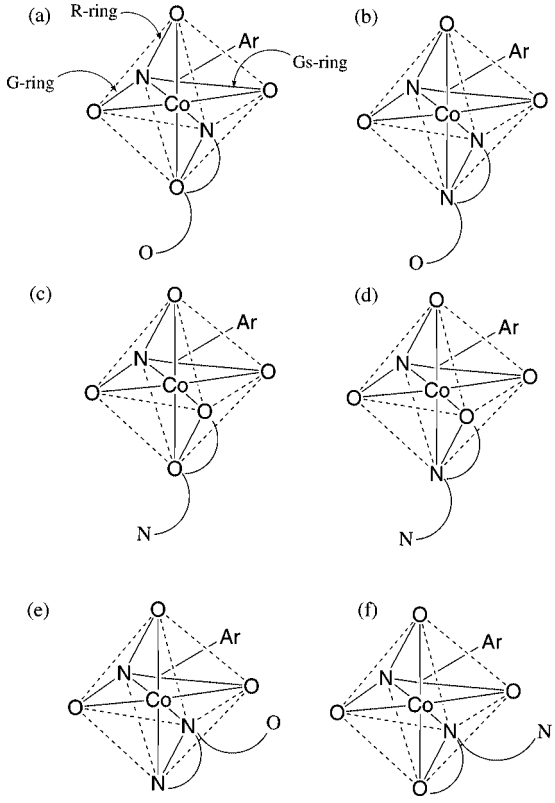

Figure 2. Six possible coordination modes of a dipeptide to a cobalt(III) complex with a tripodal NO_3-type ligand: (a) *trans*(N)-NO (coordination of N-terminal amine nitrogen *trans* to the tertiary amine of the tripodal NO_3-type ligand and the amide carbonyl oxygen); (b) *trans*(N)-NN (coordination of N-terminal amine nitrogen *trans* to the tertiary amine of the tripodal NO_3-type ligand and the amide nitrogen); (c) *trans*(O)-OO (coordination of C-terminal carboxylic oxygen *trans* to the tertiary amine of the tripodal NO_3-type ligand and the amide carbonyl oxygen); (d) *trans*(O)-ON (coordination of C-terminal carboxylic oxygen *trans* to the tertiary amine of the tripodal NO_3-type ligand and the amide nitrogen); (e) *cis*(N)-NN (coordination of the N-terminal amine nitrogen *cis* to the tertiary amine of the tripodal NO_3-type ligand and the amide nitrogen); (f) *cis*(O)-ON (coordination of C-terminal carboxylic oxygen *cis* to the tertiary amine of the tripodal NO_3-type ligand and the amide nitrogen).

Inter-Ligand Interactions Observed for the [Co(bcmpa)(dp)] Complexes

In the ^{1}H NMR spectra of the dipeptide complexes, the α-hydrogens of the Gs ring of complexes **1**–**5**, **7**, **8**, **10**, **13**, and **14**, appear at about δ = 4.8–4.9 ppm, whereas those of complexes **6** and **9** appear at about δ = 3.8–4.7 ppm. The downfield shift observed in the former complexes indicates

Table 1. ^{1}H NMR spectroscopic data of [Co(bcmpa)(dp)]$^-$ (**1**–**14**) and [Co(bcmga)(dp)]$^{2-}$ (**15**–**19**) complexes.[a]

	gly-gly **1**	gly-ala **2**	ala-gly **3**	gly-leu **4**	leu-gly **5**	gly-phe **6**	phe-gly **7**
R-ring H	3.98 (d) 4.63 (d) *J* = 18.0	3.98 (d) 4.64 (d) *J* = 18.0	3.98 (d) 4.64 (d) *J* = 18.0	4.01 (d) 4.62 (d) *J* = 18.1	3.99 (d) 4.62 (d) *J* = 18.0	3.96 (d) 4.52 (d) *J* = 18.2	3.94 (d) 4.48 (d) *J* = 17.1
G-ring H	3.20 (d) 4.13 (d) *J* = 16.8	3.22 (d) 4.15 (d) *J* = 16.5	3.22 (d) 4.11 (d) *J* = 16.5	3.40 (d) 4.29 (d) *J* = 16.5	3.20 (d) 4.11 (d) *J* = 16.5	3.39 (d) 4.20 (d) *J* = 16.3	3.37 (d) 4.19 (d) *J* = 16.7
Gs-ring H	4.90 (dd) *J* = 10.6, 4.8	4.93 (dd) *J* = 10.2, 5.2	4.87 (dd) *J* = 10.4, 4.8	4.87 (dd) *J* = 9.4, 5.4	4.87 (dd) *J* = 10.8, 4.8	4.32 (dd) *J* = 8.5, 6.4	4.80 (m)[b]
benzyl H of bcmpa (β-position of bcmga)	3.51 (dd) *J* = 15.1, 10.6 3.64 (dd) *J* = 15.1, 4.8	3.53 (dd) *J* = 15.0, 10.2 3.64 (dd) *J* = 15.0, 5.2	3.53 (dd) *J* = 15.1, 10.4 3.66 (dd) *J* = 15.1, 4.8	3.56 (dd) *J* = 15.0, 9.4 3.63 (dd) *J* = 15.0, 5.4	3.52 (dd) *J* = 15.1, 10.8 3.61 (dd) *J* = 15.1, 4.8	3.51 (m)	3.52 (m)[b] 3.59 (dd) *J* = 14.9, 3.4
N-terminal α-H of dp	4.24 (d) 4.25 (d) *J* = 17.3	4.19 (s)	4.49 (q) *J* = 7.1	4.22 (s)	4.46 (dd) *J* = 8.2, 3.0	4.02 (d) 4.14 (d) *J* = 17.3	4.73 (m)
C-terminal α-H of dp	3.82 (s)	4.10 (m)[b]	3.77 (d) 3.82 (d) *J* = 16.8	4.25 (m)[b]	3.82 (s)	4.49 (m)[b]	3.80 (d) 3.91 (d) *J* = 16.8

	gly-val **8**	gly-phg **9**	gly-D-ala **10**	gly-D-phe **11**	gly-D-phg **12**	gly-β-ala **13**
R-ring H	4.00 (d) 4.62 (d) *J* = 18.0	3.85 (d) 4.84 (d) *J* = 18.2	3.98 (d) 4.64 (d) *J* = 18.1	3.90 (d) 4.55 (d) *J* = 18.0	3.84 (d) 4.54 (d) *J* = 18.2	4.02 (d) 4.65 (d) *J* = 18.0
G-ring H	3.40 (d) 4.34 (d) *J* = 16.5	3.01 (d) 4.01 (d) *J* = 16.5	3.14 (d) 4.05 (d) *J* = 15.8	2.96 (d) 3.46 (d) *J* = 16.3	2.72 (s)	3.33 (d) 4.29 (d) *J* = 16.8
Gs-ring H	4.86 (dd) *J* = 9.5, 5.5	3.85 (dd) *J* = 10.5, 5.3	4.88 (dd) *J* = 10.1, 4.6	4.75 (dd) *J* = 10.7, 4.6	4.57 (dd) *J* = 10.7, 4.8	5.00 (dd) *J* = 10.7, 5.2
benzyl H of bcmpa (β-position of bcmga)	3.55 (dd) *J* = 15.1, 9.5 3.63 (dd) *J* = 15.1, 5.5	3.23 (dd) *J* = 15.5, 5.3 3.36 (dd) *J* = 15.5, 10.5	3.51 (dd) *J* = 15.1, 10.1 3.64 (dd) *J* = 15.1, 4.6	3.47 (dd) *J* = 15.2, 10.7 3.60 (dd) *J* = 15.2, 4.6	3.45 (dd) *J* = 15.1, 10.7 3.61 (dd) *J* = 15.1, 4.8	3.55 (dd) *J* = 15.0, 10.7 3.66 (dd) *J* = 15.0, 5.2
N-terminal α-H of dp	4.24 (d) 4.25 (d) *J* = 17.2	4.30 (d) 4.31 (d) *J* = 17.5	4.19 (d) 4.22 (d) *J* = 17.3	4.46 (d) 4.48 (d) *J* = 17.1	4.19 (d) 4.34 (d) *J* = 17.3	4.15 (d) 4.16 (d) *J* = 17.1
C-terminal α-H of dp	4.12 (d) *J* = 5.2	5.13 (s)	4.06 (m)[b]	4.49 (m)[b]	5.07 (s)	2.33 (t) *J* = 6.8

	gly-Mala **14**	gly-gly **15**[c]	gly-ala **16**[c]	gly-leu **17**[c]	gly-phe **18**[c]	gly-D-phe **19**[c]
R-ring H	3.97 (d) 4.64 (d) *J* = 18.0	4.08 (d) 4.39 (d) *J* = 18.3	4.08 (d) 4.40 (d) *J* = 18.0	4.09 (d) 4.39 (d) *J* = 18.2	4.03 (d) 4.29 (d) *J* = 18.3	4.04 (d) 4.34 (d) *J* = 18.3
G-ring H	3.21 (d) 4.05 (d) *J* = 16.5	4.40 (d) 4.76 (d) *J* = 16.8	4.39 (d) 4.69 (d) *J* = 16.7	4.44 (d) 4.80 (d) *J* = 16.7	4.36 (d) 4.72 (d) *J* = 16.5	4.33 (d) 4.47 (d) *J* = 16.2
Gs-ring H	4.93 (dd) *J* = 10.7, 4.8	4.54 (d) *J* = 6.4	4.56 (t) *J* = 6.7	4.62 (t) *J* = 6.7	4.22 (m)[b]	4.50 (m)[b]
benzyl H of bcmpa (β-position of bcmga)	3.61 (dd) *J* = 15.0, 10.7 3.69 (dd) *J* = 15.0, 4.8	2.46 (m)[d]	2.46 (m)[d]	2.47 (m)[d]	2.39 (m)[d]	2.46 (m)[d]
N-terminal α-H of dp	4.13 (d) 4.14 (d) *J* = 17.4	4.27 (s)	4.22 (s)	4.26 (s)	4.04 (d) 4.19 (d) *J* = 17.1	4.04 (d) 4.19 (d) *J* = 17.1
C-terminal α-H of dp	–	3.83 (s)	4.13 (q) *J* = 7.3	4.26 (m)[b]	4.54 (dd) *J* = 10.7, 4.0	4.50 (m) [b]

[a] Chemical shifts (δ) given in ppm relative to DSS in D_2O; coupling constants (J) given in Hz. [b] An accurate value could not be obtained because of overlap with other peaks. [c] [Co(bcmga)(dp)]$^-$. [d] β-H of the bcmga ligand.

a hydrogen-bonding interaction between the α-hydrogen of bcmpa and the coordinating carbonyl oxygen of the dipeptide. This interaction, which is mediated by the cobalt(III) complex with a bcmpa ligand, has also been observed in the crystal structure of amino acid complexes, in which the atomic distances between the α-carbon of bcmpa and the carboxyl oxygen of amino acid in *trans*(N)-[Co(bcmpa)-(leu)]$^-$ are 2.83 and 2.84 Å.[22] These data are within the range for a hydrogen bond.[32] In complex **6**, however, the corresponding signal appears at δ = 4.32 ppm, which signifies an upfield shift relative to that for **1** (δ = 4.90 ppm) with no aromatic side-chain. Some α-hydrogens of the Gs-ring in the [Co(bcmpa)(dp)]$^-$ complexes with a C-terminal aromatic amino acid are detected in the high-field region despite the downfield shift caused by the hydrogen bond described above. This is due to the ring current effect of the benzene ring. The larger upfield shift in complex **9** indicates that the benzene ring of the C-terminal phenylglycine residue approaches the α-position more closely in solution. In addition, the G-ring methylene hydrogens in **11** and **12**, which have a C-terminal aromatic D-amino acid residue, appear at δ = 2.7–3.4 ppm. The highest values of all [Co(bcmpa)(dp)]$^-$ complexes employed here indicate that the aromatic ring of the D-amino acid at the C-terminus of the dipeptide in **11** and **12** approaches the α-position of the bcmpa Gs-ring, which is interpreted in terms of an attractive inter-ligand CH–π interaction.[33] We consider that the CH–π interaction between the aromatic side-chain and the chelate ring methylene group assembles the aromatic rings around the outer coordination sphere of the ternary complex.[33,34]

To confirm the inter-ligand interactions, solvent effects on the ^{1}H NMR spectra of some complexes were examined. The α-hydrogen of **6** appears at δ = 4.40 ppm in less polar solvent (75% [D_8]dioxane and 25% D_2O) and at δ = 4.32 ppm in more polar solution (D_2O). Notably, all the other hydrogens of **6** appear in the up-field region under less polar conditions. In the cases of **1** and **4**, which have no C-terminal aromatic ring, all hydrogens appear in the upper field region under less polar conditions than those provided by D_2O. Hydrophobic interaction such as the CH–π interaction that is present in the aqueous phase does not form under less polar conditions.[33,34] The results are listed in Table 2.

Solvent effects were also investigated by CD spectroscopy. In complexes **1**, **13**, and **14**, whose only asymmetric ligand is bcmpa, no CD spectral variations were observed between polar (water) and less polar (water/dioxane = 1:3) solvents. In the case of **4**, which contains bcmpa and an asymmetric C-terminal bulky aliphatic group, the CD intensity at about 16×10^3 cm^{-1} in water increases and that at 20×10^3 cm^{-1} decreases upon addition of dioxane, as shown in Figure 3 (a). Such a spectral change is caused by a conformational change of the asymmetric ligand bound to the cobalt(III) center. On the other hand, in the case of **6**, which contains bcmpa and an asymmetric C-terminal aromatic group, the CD intensities at about 16 and 20×10^3 cm^{-1} gradually decrease upon addition of dioxane to the aqueous solution, as shown in Figure 3 (b). These findings are interpreted in terms of a hydrophobic inter-ligand interaction between the C-terminal aromatic side-chain of the dipeptide and the benzene ring of bcmpa in the ternary complexes.

We have previously reported that three inter-ligand interactions, such as hydrogen bonds, steric repulsion, and electrostatic interactions, regulate the coordination structure of the ternary cobalt(III) complexes [Co(bcmpa)(aa)]$^-$.[22,24] Two kinds of hydrogen bond in the ternary complexes employed here act between N-terminal amino hydrogens of the dipeptide and carboxyl oxygen atoms of bcmpa and between the α-hydrogen of bcmpa and the amide carbonyl group of the dipeptide. The hydrogen bond plays an important role in the recognition of the dipeptide N-terminus because it supplies a large fraction of energy required for stabilization of the transition state of an enzyme reaction.[35] Steric repulsion of the hydrogen atoms between the methylene groups on the G- or Gs-chelate rings of bcmpa and the N-terminal amino group is expected to promote the formation of the *trans*(N) geometry. An electrostatic interaction is expected to inhibit the formation of the *cis*(N) one because it acts repulsively between the negative charge of the two carboxylate moieties. From the above discussion, which is summarized in Figure 4(a), a ternary cobalt(III) complex coordinated by the N-terminal nitrogen of the dipeptide and bcmpa with a *trans*(N) geometry is formed preferentially. This is an interesting model for mimicking aminopeptidase, even though it is a low-molecular compound. In the case of complexation of a dipeptide having a C-terminal aromatic side-chain, the inter-ligand CH–π interaction assembles the benzene rings of the dipeptide and bcmpa to form a hydrophobic sphere, as shown in Figure 4(b). Although we were not able to obtain a crystal of [Co(bcmpa)(dp)]$^-$, the coordination structures in Figure 4 are proposed based on the above spectral considerations.

Table 2. Solvent effect on the proton chemical shift value in typical [Co(bcmpa)(dp)]$^-$ complexes.[a]

	gly-gly (**1**)				gly-leu (**4**)				gly-phe (**6**)			
Ratio water/dioxane	100:0	75:25	50:50	25:75	100:0	75:25	50:50	25:75	100:0	75:25	50:50	25:75
R-ring H	3.98, 4.63	3.92, 4.59	3.84, 4.45	3.78, 4.45	4.01, 4.62	3.93, 4.54	3.86, 4.46	3.80, 4.41	3.96, 4.52	3.90, 4.44	3.83, 4.39	3.76, 4.36
G-ring H	3.20, 4.13	3.18, 4.10	3.13, 4.06	3.11, 4.05	3.40, 4.29	3.37, 4.28	3.36, 4.28	3.36, 4.30	3.39, 4.20	3.36, 4.18	3.35, 4.17	3.30, 4.17
Gs-ring H	4.90	4.86	4.82	4.79	4.87	4.81	4.76	4.73	4.32	4.31	4.33	4.40

[a] Chemical shifts (δ) given in ppm relative to DSS in D_2O.

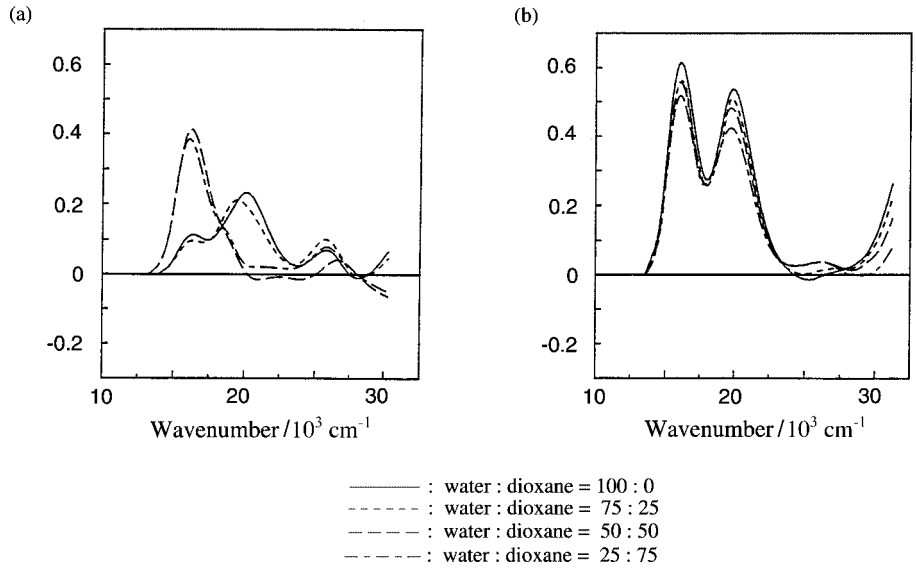

Figure 3. Solvent effects on the inter-ligand interaction between the cobalt(III) ternary complexes and the dipeptide ligand observed by CD spectroscopy: (a) $[Co(bcmpa)(gly\text{-}leu)]^-$ (**4**); (b) $[Co(bcmpa)(gly\text{-}phe)]^-$ (**6**).

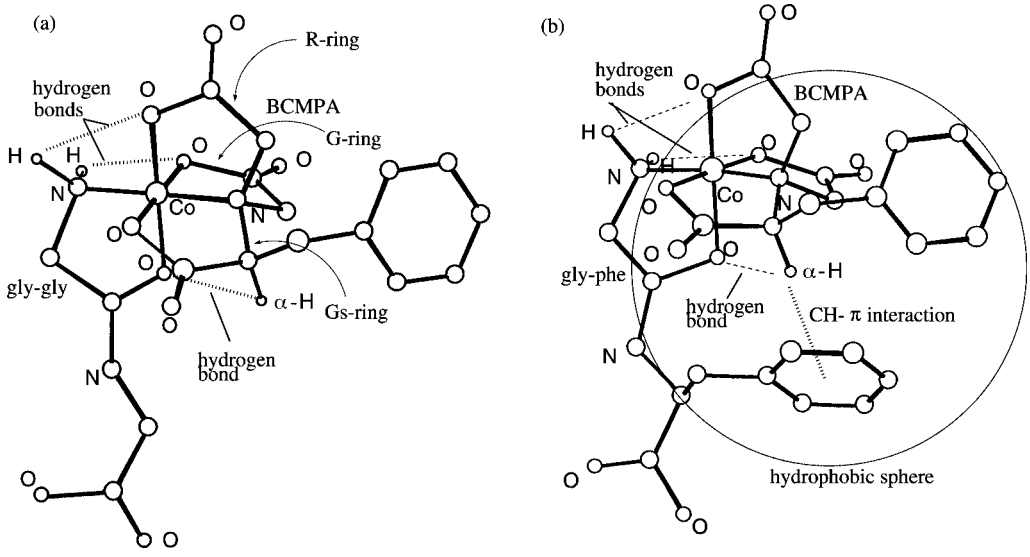

Figure 4. Schematic drawing of the inter-ligand interactions in the ternary complex: (a) $[Co(bcmpa)(gly\text{-}gly)]^-$ (**1**); (b) $[Co(bcmpa)(gly\text{-}phe)]^-$ (**6**), showing the hydrophobic sphere.

Hydrolysis of Dipeptide Ligands Mediated by the Ternary Cobalt(III) Complexes

cis-Diaquacobalt(III) complexes are known to hydrolyze carbonyl esters, amides, nitriles, and phosphate esters by the coordination of these substrates to the metal center.[36] Buckingham et al. have shown that the hydrolysis of amino acid esters and peptides is accelerated 106 times in the presence of a cobalt(III) complex of a linear tetraamine ligand in neutral media.[17,37] These reactions are promoted by the nucleophilic attack of hydroxide at the amide carbonyl group activated by coordination to the metal center. Under these reaction conditions, however, selectivity for the cleavage reaction was not reported.[38] Since the tetraamine ligand has no functional groups for recognition of the peptide, it could not show any selectivity. Exceptionally, a β-$[Co(trien)(OH)(H_2O)]^{2+}$ complex has been reported to show selective hydrolysis of peptides[5] even though no inter-ligand interactions were observed in this complex. Here, we have designed ternary cobalt(III) complexes containing a bcmpa ligand, which is expected to regulate the selective hydrolysis of dipeptides due to its having a recognition site for the aromatic group. From the viewpoint of the selective cleavage of peptide bonds, a ternary cobalt(III) complex having inter-ligand interaction sites becomes a molecular recognition model for aminopeptidases. In order to determine the active species for the hydrolysis of the amide bond, we carried out the reaction with $[Co(bcmpa)(leu\text{-}gly)]^-$ (**5**) under different pH conditions at 40 °C. The yield of the

$[Co(bcmpa)(leu)]^-$ complex generated upon hydrolysis of the leu-gly moiety of **5** was higher at higher pH, as shown in Figure 5. This finding indicates that the reaction is promoted by OH^- ion. Since the amount of unidentified cobalt(III) complexes increased in the higher pH region, all reactions were carried out at pH 9.

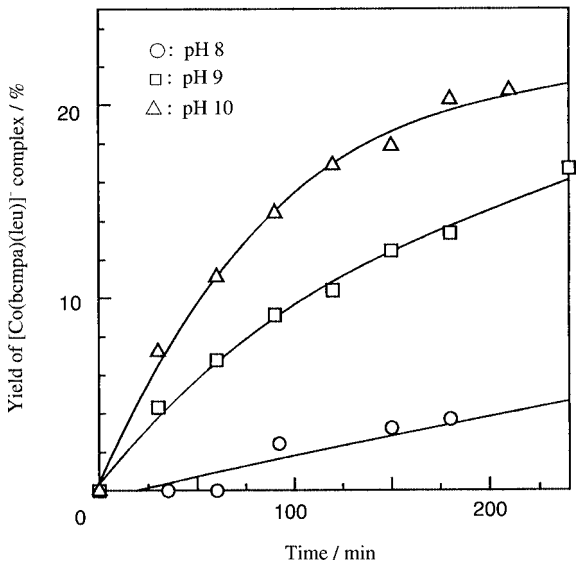

Figure 5. Rate of formation of $[Co(bcmpa)(leu)]^-$ during the hydrolysis of $[Co(bcmpa)(leu\text{-}gly)]^-$ (**5**) and its dependence on pH.

The yields of the cleavage product, $[Co(bcmpa)(aa)]^-$, at the initial stage of the reaction are listed in Table 3. The most striking observation in these reactions is the complete inhibition of hydrolysis of complexes **6** and **11**. The $[Co(bcmpa)(gly)]^-$ complex was not detected even after reaction of **6** and **11** at pH 9.0 and 40 °C for 3 h. This finding is in contrast to a previous report that Co-trien-dp complexes can be used for cleavage reactions of peptides including C-terminal aromatic amino acid residues.[17] The hydrolysis of complexes **1** and **2**, which have a C-terminal glycine or alanine residue, proceeded easily, as shown in Figure 6. On the other hand, the yield of $[Co(bcmpa)(gly)]^-$ from the reaction of **4** and **8**, which have C-terminal bulky dipeptides such as gly-val and gly-leu, was poor. The hydrolysis rate for complex **1** was fivefold higher than those for **4** and **8**. Upon increasing the steric bulkiness of the side chain of the dipeptide at the C-terminus, the yield of the hydrolysis product decreased according to the order glycine (C-0), alanine (C-1), valine (C-3), and leucine (C-4). The reaction of the dipeptide complexes having C-terminal aromatic side-chain (**9** and **12**) did not give the corresponding amino acid complexes, and neither did the complex with C-terminal phenylalanine. In contrast to the above findings, the reaction of **5**, which has an N-terminal bulky substituent, gave $[Co(bcmpa)(leu)]^-$ in moderate yield. In the case of complex **14**, which has an α,α-disubstituted amino acid at the C-terminus, the yield of the $[Co(bcmpa)(gly)]^-$ complex was only 1.7% after 3 h; this was also interpreted in terms of a steric effect.

Table 3. Reactivity for the cleavage reaction of various dipeptides at the ternary cobalt(III) complexes.[a]

Cobalt(III) complexes		Yield of gly complex[b] (%)
bcmpa	gly-gly (**1**)	24.9
	gly-ala (**2**)	16.0
	gly-leu (**4**)	3.9
	leu-gly (**5**)	13.4[c]
	gly-phe (**6**)	0.0
	gly-val (**8**)	5.1
	gly-phg (**9**)	3.7
	gly-D-ala (**10**)	17.3
	gly-D-phe (**11**)	0.0
	gly-D-phg (**12**)	2.0
	gly-Mala (**14**)	1.7
bcmga	gly-gly (**15**)	26.3
	gly-ala (**16**)	8.9
	gly-leu (**17**)	2.2
	gly-phe (**18**)	2.9
	gly-D-phe (**19**)	4.6

[a] The reaction was performed on a 1.25×10^{-5} mol scale in an aqueous solution of boric buffer (pH 9) at 40 °C for 3 h. [b] Yield of $[Co(bcmpa)(gly)]^-$ or $[Co(bcmga)(gly)]^{2-}$ determined by HPLC analysis based on the starting complexes $[Co(bcmpa)(dp)]^-$ or $[Co(bcmga)(dp)]^{2-}$. [c] $[Co(bcmpa)(leu)]^-$.

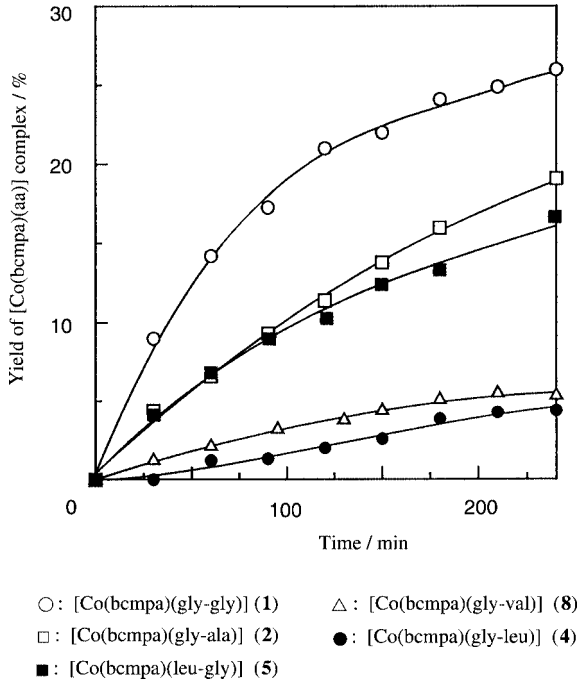

Figure 6. Cleavage reactions of various Co-bcmpa-dp complexes.

Two plausible mechanisms for the hydrolysis of amide compounds mediated by the mononuclear cobalt(III) complex have been proposed.[17,39] One involves external attack of OH^- species on the amide carbonyl group, which is activated by coordination to the metal center,[40] and the other involves internal attack of the OH^- species, which is activated by coordination to the metal center where the amide carbonyl group is coordinated.[41] In the cases of metalloen-

zymes containing a mononuclear active site, the internal attack mechanism has been proposed on the basis of the X-ray crystal structural analysis of carboxypeptidase A.[20] However, the ternary cobalt(III) complexes employed here have no coordination site available for the OH^- species, as illustrated in Figure 4, therefore hydrolysis of the dipeptide must occur externally. With bcmpa as ligand hydrolysis of **6** and **11** did not give the corresponding amino acid, in contrast to the reaction with bcmga as ligand. As bcmga has a carboxylate moiety instead of the phenyl moiety of bcmpa, this makes for a more hydrophilic coordination sphere. In the hydrolysis reactivity of dipeptide complexes containing a C-terminal aliphatic side-chain, no difference was found between the bcmpa and bcmga ligands (Table 3). In Figure 7, however, the reactivity of complexes **6**, **11**, **18**, and **19**, which have C-terminal aromatic side-chains is clearly not equal; the dipeptide complexes with bcmga give small amounts of the corresponding cleavage products whereas those with bcmpa give no cleavage products. In the case of the bcmpa system, the hydrophobic interaction around the outer coordination sphere [Figure 4 (b)], which is generated by the assembly of the benzene ring of bcmpa and the C-terminal side-chain of the dipeptide, inhibits the approach of OH^- to the amide carbonyl group. In the case of bcmga, however, such a hydrophobic sphere is not formed around the complex. Although the overall charges of the complexes Co-bcmpa (–1) and Co-bcmga (–2) are different, the effect of the charge on acceleration of the cleavage reaction is not yet clear. The more negatively charged $[Co(bcmga)(dp)]^{2-}$ complex and the carboxylate moiety attached to bcmga seems to attract hydrophilic OH^- species externally. It is interesting that such a simple complex is able to perform the sequence-specific cleavage of peptides by a combination of inter-ligand interactions. We have also reported the selective cleavage of various peptides using simple enzyme model complexes in which the bulkiness of the aliphatic side-chain in the ternary cobalt(III) complex with a terpy ligand controls the specificity of the oxidative degradation of peptides.[42]

In summary, some ternary cobalt(III) complexes ($[Co(bcmpa)(dp)]^-$) containing an asymmetric tripodal ligand and dipeptides have been prepared. Their coordination geometry has been determined by UV/Vis, CD, and ^1H NMR spectroscopic methods. Site-specific recognition of dipeptides occurs by various kinds of weak non-covalent inter-ligand interactions. Such interactions are also observed in the ternary complexes with an amino acid ligand, $[Co(bcmpa)(aa)]^-$, some of whose structures have been determined by X-ray crystallography. Under slightly alkaline conditions, the dipeptide ligand of the ternary complexes is cleaved to give $[Co(bcmpa)(aa)]^-$. Selectivity for hydrolysis of the dipeptide ligand is regulated by the above interactions. This is the first report describing selectivity for the cleavage of various peptides using simple enzyme model complexes. The hydrophobic interaction between bcmpa and the C-terminal side-chain of the dipeptide regulates the approach of OH^- to the amide carbonyl group coordinated to the cobalt center. In particular, the CH–π interaction caused by assembly of the benzene ring of bcmpa and the C-terminal aromatic amino acid moiety inhibits the approach of OH^- and prevents hydrolysis of the dipeptide. The present ternary (Co-bcmpa-dp) system provides a method for the selective cleavage of peptide compounds due to multi-site interactions that is superior to the previous ternary (Co-trien-dp) system without the recognition site. We conclude that the multi-site interactions mediated by the metal complexes could be used to design an artificial hydrolysis enzyme.

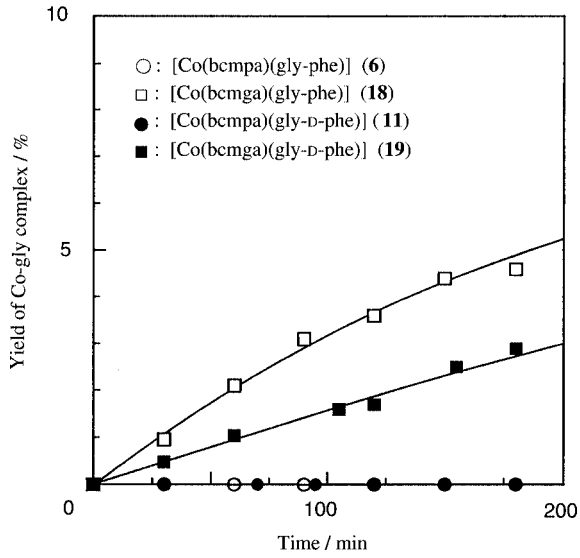

Figure 7. Cleavage reaction of cobalt(III) complexes having a C-terminal aromatic side-chain.

Experimental Section

Material: The NO_3-type tripodal tetradentate ligand H_3bcmpa, was prepared from L-phenylalanine by a literature procedure.[22,24] H_3bcmga [bis-*N*,*N*-carboxymethyl-L-glutamic acid], which is a coordination analogue of bcmpa, was prepared in a similar manner from L-glutamic acid. QAE Sephadex A-25 (Cl^- type) was supplied by Pharmacia. All amino acid derivatives (L- and D-isomers) and gly-gly (grade AA) were obtained from Peptide Institute Inc. and used without further purification. Other dipeptide compounds, namely gly-ala, ala-gly, gly-leu, leu-gly, gly-phe, phe-gly, gly-val, gly-phg, gly-D-ala, gly-D-phe, gly-D-phg, gly-β-ala, and gly-Mala, were synthesized by a DCC coupling method from the corresponding protected amino acid derivatives.

Measurements: The UV/Vis spectra were recorded with a JASCO UVDEC-660 spectrophotometer. The circular dichroism (CD) spectra were recorded with a JASCO J-500C spectropolarimeter. All measurements for the ternary cobalt(III) complexes were carried out in water and/or water–dioxane solution at room temperature. ^1H NMR spectra were recorded with a JEOL JNM-FX-400 spectrometer in D_2O and/or D_2O/[D_8]dioxane solvent, with DSS as an internal standard. The HPLC analytical system was constituted by a JASCO Gulliver Series with a finepak SIL NH_2-5 column. The analytical conditions of HPLC were as follows: the mobile

phase (flow rate: 1 mL min^{-1}) was a 2:8 mixture of methanol and $NaHPO_4$ buffer (0.05 M) and the product complexes were monitored with a UV/Vis detector (wavelength: 510 nm).

Preparation of Ternary Cobalt(III) Complexes with a Dipeptide Ligand: K[Co(bcmpa)(gly-gly)] was prepared in a manner analogous to K[Co(bcmpa)(aa)].[22,24] An equimolar amount of gly-gly and 0.05 g of active charcoal were added to 10 mL of an aqueous solution of K_2[Co(bcmpa)(CO_3)] (0.2 M). The resulting mixture was stirred for 24 h at 40 °C with addition of dilute HCl to maintain the pH at 6–7. After filtration of active charcoal and desalting with methanol, the resulting mixture was poured onto a QAE Sephadex A-25 column (Cl^- form, 2.5 × 35 cm). The cobalt(III) complexes adsorbed on the anion exchange column were washed with distilled water to remove neutral and cationic complexes, and then two negatively charged bands were separated with KCl solution (0.1 M). The orange-colored first eluting band was the main fraction, which gave 0.49 g of product complex (49% yield) after usual isolation, and the pink-colored second one was the minor product (0.05 g, 5% yield). On the basis of the spectroscopic analysis, the former was identified as the dipeptide complex K[Co(bcmpa)(gly-gly)] (**1**) and the latter as the amino acid complex K[Co(bcmpa)(gly)]. Other negatively charged bands were also eluted in this separation, but they were unable to be identified because of lack of product.

K[Co(bcmpa)(gly-gly)] (1): ^{1}H NMR (D_2O): δ = 3.20 (d, J = 16.8 Hz, 1 H, G-ring-H),[30] 3.51 (dd, J = 15.1 and 10.6 Hz, 1 H, benzyl-H of bcmpa), 3.64 (dd, J = 15.1 and 4.8 Hz, 1 H, benzyl-H of bcmpa), 3.82 (s, 2 H, C-terminal glycine α-H), 3.98 (d, J = 18.0 Hz, 1 H, R-ring-H), 4.13 (d, J = 16.8 Hz, 1 H, G-ring-H), 4.24 (d, J = 17.3 Hz, 1 H, N-terminal glycine α-H), 4.25 (d, J = 17.3 Hz, 1 H, N-terminal glycine α-H), 4.63 (d, J = 18.0 Hz, 1 H, R-ring-H), 4.90 (dd, 10.6 and 4.8 Hz, 1 H, Gs-ring-H), 7.4–7.6 (m, 5 H, aromatic-H).

The other K[Co(bcmpa)(dp)] complexes **2**–**14** were prepared by the same procedure using the appropriate dipeptide instead of gly-gly. The K_2[Co(bcmga)(dp)] complexes **15**–**18** were prepared from K_3[Co(bcmga)(CO_3)]. Their ^{1}H NMR spectroscopic data are listed in Table 1.

Hydrolysis of Dipeptide Ligands: Hydrolysis of dipeptide ligand mediated by the cobalt complexes was carried out as follows. K[Co(bcmpa)(dp)] or K_2[Co(bcmga)(dp)] (about 0.07 g, 1.25×10^{-5} mol) was dissolved in 50 mL of an aqueous solution of 0.1 M H_3BO_3/Na_2HBO_3 buffer, which was maintained at pH 9.0 and 40 °C. The ionic strength during the reaction was 1.0 M of KCl. An aliquot of the reaction mixture passed through a 0.45-μm filter was analyzed by HPLC at 30 min intervals. After 3 h, the reaction solution was treated with the same isolation method as described above. Mono-negative charged complexes generated by the reaction after QAE Sephadex column separation with 0.1 M KCl solution were characterized by ^{1}H NMR, UV/Vis, and CD spectroscopy.

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